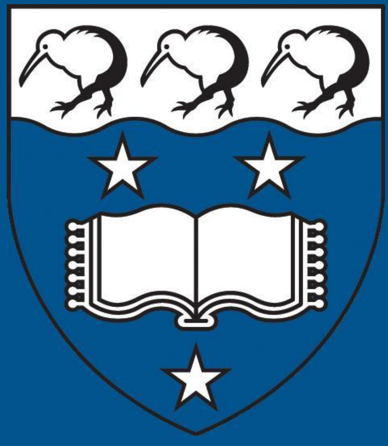


# Effect of Light Regimes on the Utilisation of an Exogenous Carbon Source by Freshwater Biofilm Communities

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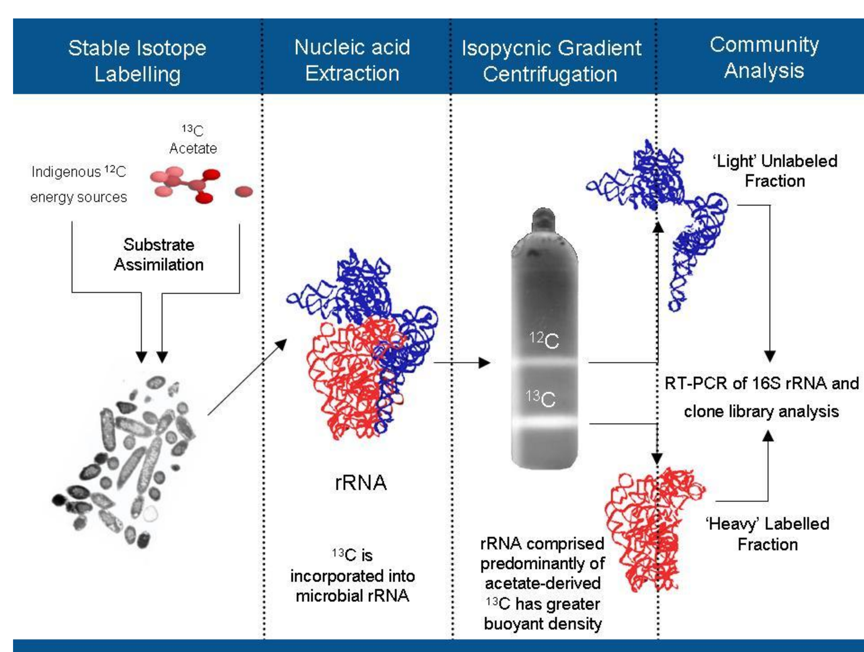
## Introduction

Biofilms are a major source of 'in stream' primary production, exhibiting high population density, and being an important source of carbon for microbial heterotrophs. Changing patterns of land-use within freshwater catchment areas may greatly impact on stream biofilm microbial community structure, which in turn may influence the speed and extent with which full ecosystem recovery may occur.

This study reports how freshwater biofilm communities respond to additions of acetate, used as a proxy for organic matter, the primary source of carbon within shaded forests and headwater streams. The use of [$^{13}$C] acetate and subsequent isolation of $^{13}$C-labelled nucleic acids from the metabolically active fraction of the bacterial community enabled substrate assimilating organisms to be identified. In addition, biofilms were exposed to varied levels of incident light to assess the relative contribution of phototrophic and heterotrophic nutrition.

## Nucleic Acid Stable Isotope Probing

The incorporation of a stable-isotope-labeled substrate (i.e. $^{13}$C-sodium acetate) into cellular biomarkers, including nucleic acids, enables substrate assimilating organisms to be identified, via stable-isotope probing (see diagram below).

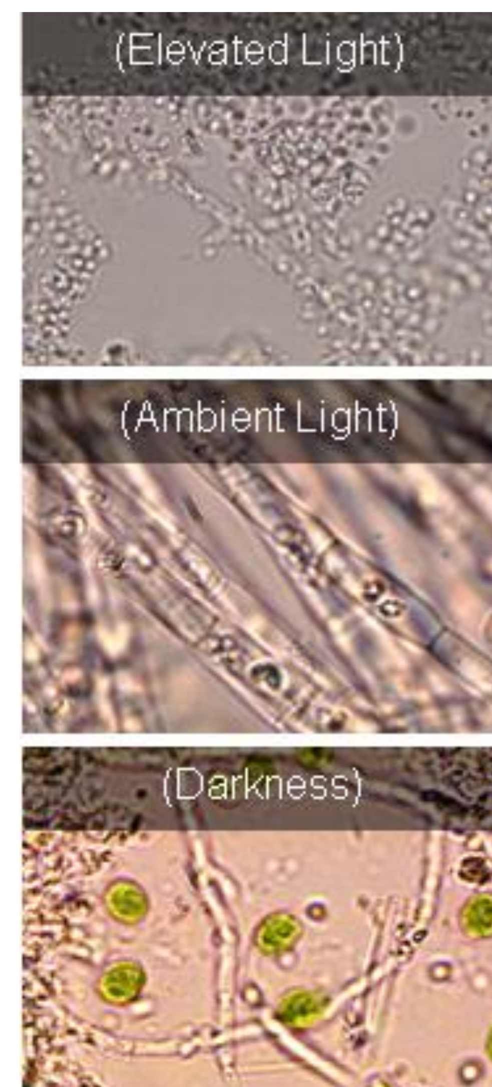



## Experimental Design

Nine identical slide rack microcosms were constructed and standard microscopy slides (five per microcosm) were incubated under natural light for six weeks in stream water to culture biofilm communities. Treatments were then constructed in triplicate consisting of microcosms incubated in (a) ambient light, (b) absolute darkness (c) elevated light, exhibiting 50% greater irradiance than the 'ambient light' treatment. Following seven days incubation, microcosms were augmented with 2 mM $^{13}$C-sodium acetate (final concentration). Over 21 d incubation, assessments were made of changes in biofilm biomass, bacterial and fungal heterotrophic counts and mass of chlorophyll. Molecular analysis of the microcosm communities of both the 'standard' and $^{13}$C-labelled nucleic acids targeted a conserved region of bacterial 16S rRNA and rDNA to identify substrate assimilating organisms.

## Results

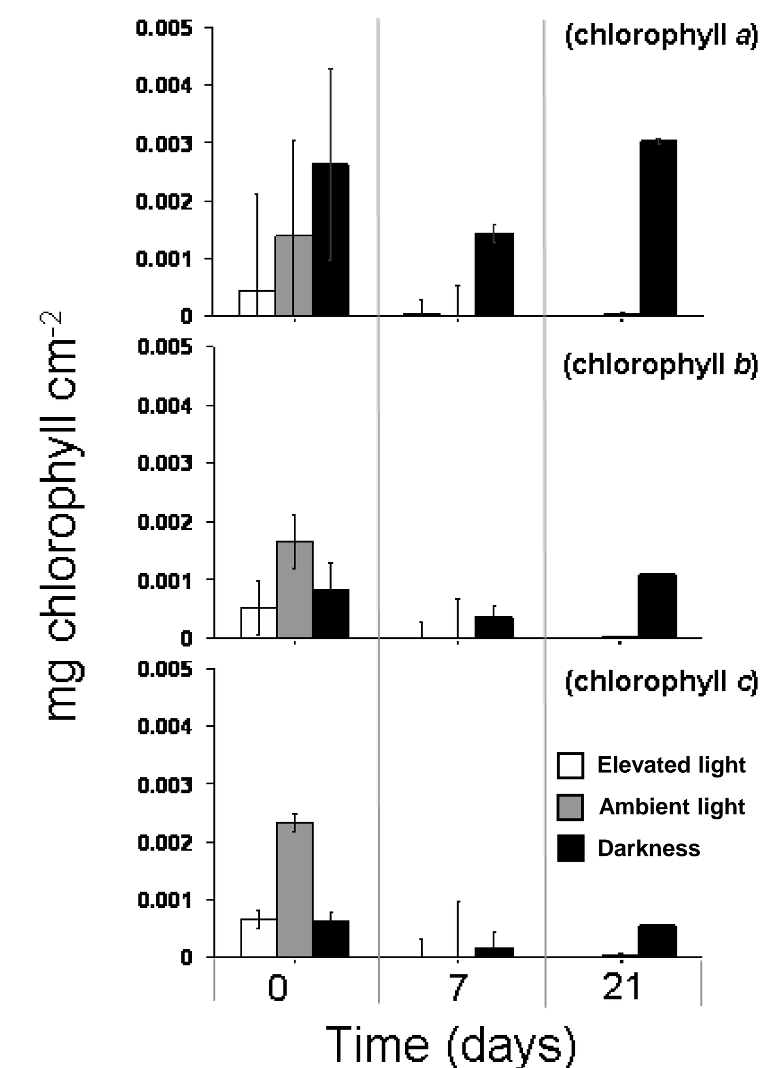
### Biofilm Structure




Microscopy revealed the development of a distinct biofilm structure after 13 d incubation as individual treatments under modified light regimes (see figure on left).

- Elevated light – No visible green organelles
- Ambient light – Dominated by algal strands
- Dark – Dominated by algal strands and green single-celled organisms

### Biofilm Chlorophyll Content



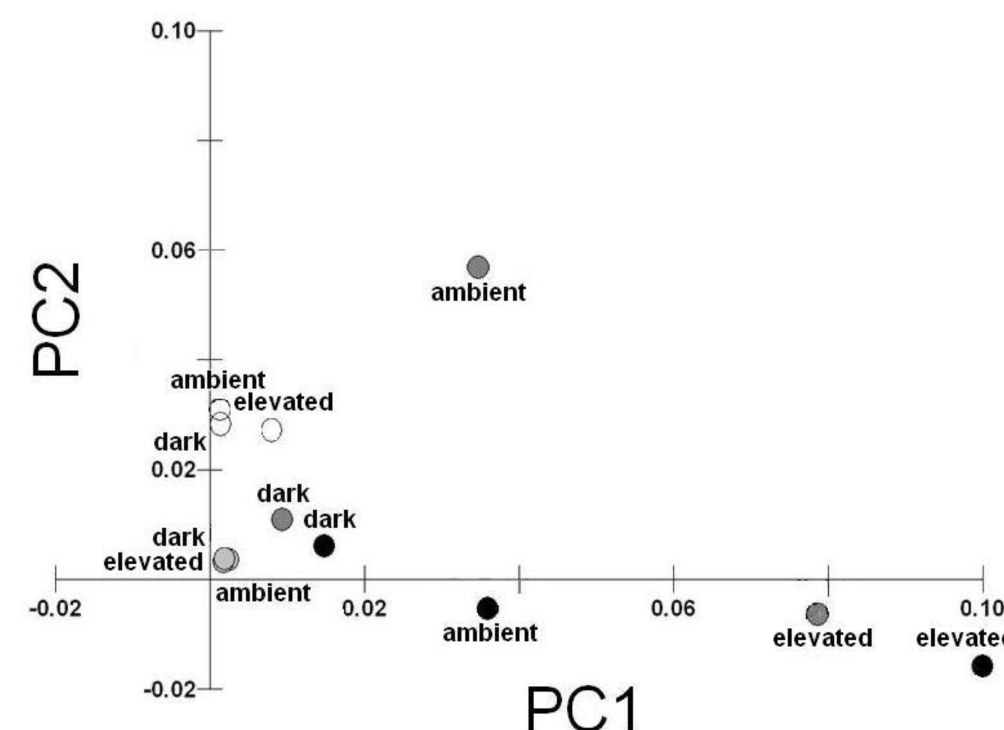

In support of microscopy analysis, concentrations of chlorophyll declined least within the dark treatment. No chlorophyll was detected within the elevated and ambient light treatments after 7 d incubation (see figure on left).

A possible cumulative effect between the addition of acetate and increased cellular photoinhibition is proposed.

### Molecular Analysis

#### Bacterial Community Profiles

Multivariate Principal Component Analysis for ARISA profiles of bacterial biofilm communities sampled from treatments incubated in elevated light, ambient light and darkness at time -7 d (○); 0 d (●); 7 d (●); 21 d (●). In this analysis, the variation between the datasets is projected onto two axes, in this case PC1 and PC2, which account for 35% and 21% of the total variability. Points located close together are presumed to represent similar datasets.

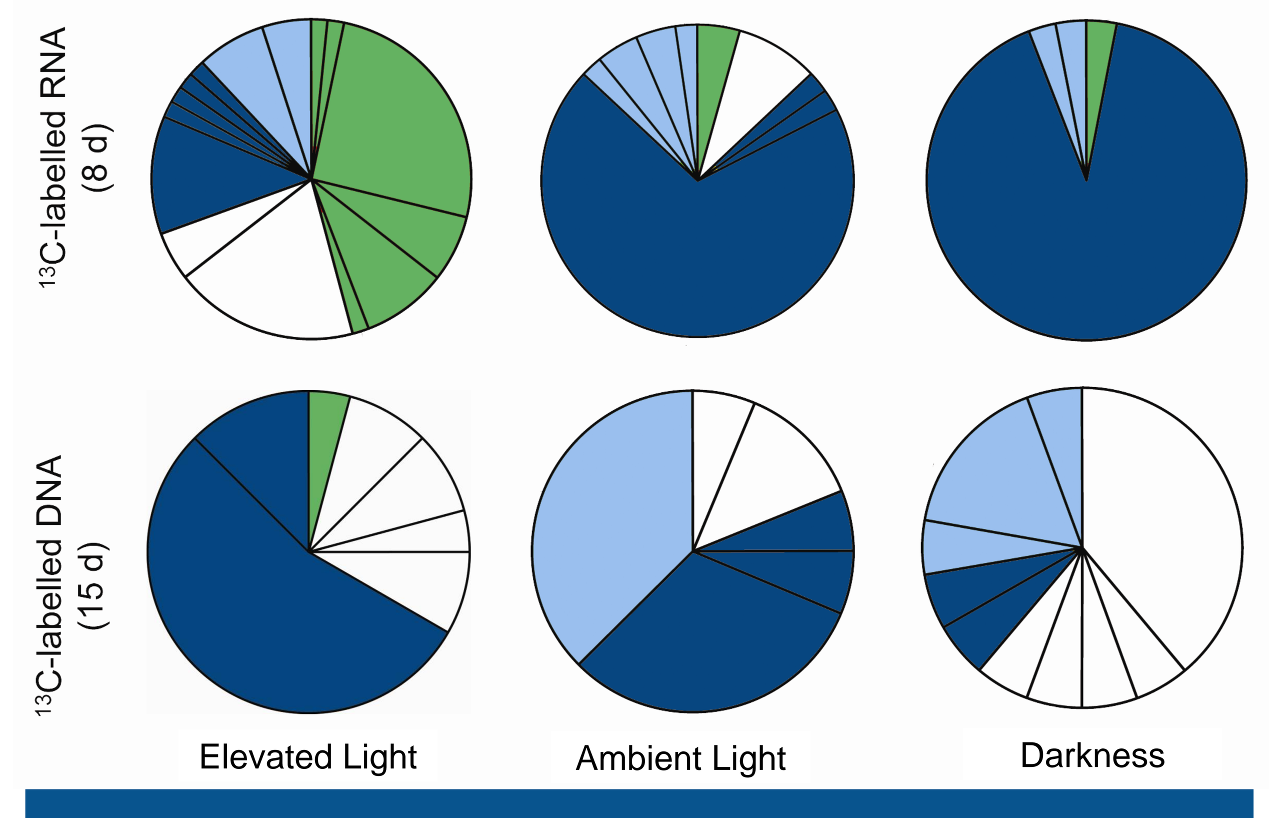



- Clone library assessment of the bacteria actively utilising $^{13}$C-acetate revealed a very different microbial community developed within dark incubated treatments (8 and 15 d; see figure on right). This is in contrast to ARISA community profiles and suggests that greater change occurred within the community actively incorporating $^{13}$C as compared to the total population.
- The β-proteobacteria became the dominant phylum of clones isolated from the $^{13}$C-labelled DNA within dark incubated treatments (T = 15 d), predominantly phylotypes related to Mn-oxidising and Fe-reducing bacteria
- Cyanobacterial rRNA, found in the initial biofilm community (T = 0 d), was not detected following the addition of acetate, indicating a shift towards a more heterotrophic community.

- Following application of different light regimes, the bacterial community structure changed least within dark incubated biofilm samples (see figure on left).

#### Bacterial Community Composition

Composition of microcosm biofilm bacteria utilising $^{13}$C-labelled acetate, determined by 16S rRNA and rDNA clone libraries. $^{13}$C-labelled nucleic acids were separated by isopycnic centrifugation. incubated under elevated light, ambient light and absolute darkness. (■) α-proteobacteria; (□) β-proteobacteria; (■) δ-proteobacteria; (■) other.




## Conclusions

(a) Bacteria actively utilising acetate, added as a proxy for organic matter differed to that of the general biofilm community, stimulated by the addition of labile carbon.

(b) Changes to light regime affected the structure of the bacterial communities actively metabolising $^{13}$C-acetate and those not incorporating the isotopic label, differently.

(c) Cyanobacterial rRNA were not detected within acetate-amended treatments. In addition, no green pigmented organisms were observed in the elevated light treatment and levels of chlorophyll were below detection. A possible cumulative effect between the addition of acetate and increased cellular photoinhibition is proposed.

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